

Research article

## Captive breeding of the Shark Bay mouse *Pseudomys fieldi* to facilitate species recovery in the wild

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### Abstract

Shark Bay mice (*P. fieldi*) were bred at Perth Zoo to provide animals for release to the wild as part of recovery actions for the species. Three-hundred and thirty-five young were produced from 93 litters, with an average litter size of 3.6 (range 1–6). Sexual maturity for both sexes was reached at 65 days of age, and breeding was observed all year round. The oldest female to give birth was 625 days of age and the oldest male to sire young was 531 days of age. Following a planned interruption to the programme and the separation of breeding pairs, there was some difficulty in later re-establishing reproduction. A strategy to stimulate a return to breeding, along with detailed husbandry methods, is described.

### Introduction

The Shark Bay mouse (*Pseudomys fieldi*), also known as the djoongari, is a robust (30–45 g), long-haired Australian native rodent (Watts and Aslin 1981). It is now naturally found on only one offshore island, Bernier Island, off the Western Australian coast, although the species was once found on mainland Australia across three states and territories (Western Australia, South Australia and the Northern Territory). The last specimen from the mainland was collected in 1895 near Alice Springs (Morris et al. 1996).

The population size on Bernier Island was estimated to be about 6000–7000 in 1992 (Morris et al. 2000) and due to its extremely restricted distribution was classified nationally as Endangered. In 1991, DPaW (Department of Parks and Wildlife, previously CALM – Department of Conservation and Land Management) produced a draft Recovery Plan for *P. fieldi*, and set up a recovery team to oversee its implementation. A full Recovery Plan was produced for the period 1992–2001 with a series of actions including research, reintroductions,

translocations, control of introduced predators and competitors and captive breeding identified to improve the conservation status of the species. In December 1996 a captive population was established at Perth Zoo (South Perth, WA) to breed animals for reintroduction and to supplement wild-to-wild translocations.

Prior to the establishment of the Perth Zoo breeding colony, reproductive knowledge of *P. fieldi* came from observations from one captive breeding pair (Watts and Spencer 1978) and notes from a small captive colony consisting of three pairs of wild-caught animals established at DPaW's Research Centre in Woodvale, Western Australia. Six months after establishment of the Woodvale colony, five of the founders and all 16 of their offspring were transferred to Perth Zoo so breeding could continue on a larger scale. The colony was maintained until late 2002, with an additional 17 (10 male and 7 female) founders from the wild being added over that period to broaden the genetic mix.

As various rodent species in captivity can display aggression towards mates and have been known to cannibalise and

abandon their pups (Smith et al. 1972; Watts and Aslin 1981; Elwood 1983), it was decided to adopt a minimally invasive approach to their management. This strategy continued until 2001, when the captive programme began to wind down after Recovery Team production targets were met. It was then decided to allow access to the colony for research purposes before it was completely disbanded. Butland (2001) completed a study to clarify reproductive parameters and determine whether increased handling affected breeding.

Under the IUCN Red List of Threatened species, *P. fieldi* is currently classified as Vulnerable (Morris and Richards, 2008).

## Housing

On Bernier Island *P. fieldi* is most commonly found in coastal sandy areas (Morris et al. 2000) dominated by *Spinifex longifolius*, *Olearia axillaris* and scattered salt-bushes – *Atriplex paludosa* and *Rhagodia obovata* (Robinson et al. 1976). It is also found in *Triodia/Acacia* heath (Morris et al. 2000), and has been observed to use beach-cast sea-grass as a refuge (Robinson 1983). Indoor enclosures were fitted out to replicate these wild habitats as closely as possible within the obvious constraints of captivity such as restricted space, artificial lighting, and minimal exposure to natural environmental cues.

Animals were maintained in an air-conditioned transportable building under the natural day length conditions for Perth, Western Australia (31° 57'S, 115° 52'E) with temperatures ideally maintained between 20° and 27° C. Enclosures were 1.2 m glass aquaria with metal-meshed lids and a substrate of white washed sand covered with a thick layer of sea-grass to mimic the sandy coastal areas where *P. fieldi* can be found in the wild. Thick layers of *Olearia axillaris* and *Acacia* sp. branches were added to the enclosures to give the animals a sense of security (cover), and to provide enrichment. The hard stems of the *Acacia* sp. provided opportunities for the mice to chew and maintain teeth and gum health, and leaves were removed by the mice to line nests. The sand, sea-grass and branches were mixed together by the animals to create runways, tunnels and nesting sites. Finch nest boxes and 60–90 mm diameter PVC pipe cut into short lengths (approximately 200 mm) were also provided as additional nest sites, and were often entwined and half buried by the mice within the substrate. The importance of providing natural conditions within enclosures to stimulate breeding was recognised by Happold (1976), who describes successful breeding of other *Pseudomys* species, and we followed this premise where possible.

Breeding pairs were generally housed in two adjoining tanks with access provided between them via 65 mm diameter PVC pipes, with flanges and removable metal slides covering holes cut in the ends of the tanks.

Stainless steel feed and water bowls were secured to the glass with silicone to reduce spillage.

## Diet

*P. fieldi* appear to be omnivorous (Morris et al. 2000). Scat analysis from a handful of animals on Bernier Island revealed leaf fragments of *Olearia*, and flower parts possibly from the same plant. Leaves and stem parts from a fleshy dicot were also found, as well as insect fragments (Robinson et al. 1976). They have also been seen eating spiders (Morris et al. 2000).

In captivity, *P. fieldi* was fed a staple diet of a 50:50 combination of budgie seed and pigeon mix on a daily basis. This was supplemented and varied by the addition of fruit and vegetables (sweet potato, apple or carrot), fresh fodder, rodent cubes, seeding grasses and sunflower seeds on different days. Fresh fodder included *Spinifex longifolius*, various species of salt-bush,

sea-spinach (*Tetragonia decumbens*) and pig-face (*Carpobrotus virescens*). All items were variously used for food or nesting material. We considered it important to include items that would be found in the environment of the proposed release sites so that upon release the mice would be immediately familiar with available food, particularly the fleshy vegetation that would provide them with a vital source of moisture.

As *P. fieldi* is nocturnal, food was placed in enclosures late in the day, so the vegetable/plant component remained fresh.

## Routine handling and monitoring

Single animals were sighted every three days and weighed fortnightly to monitor health and body condition. Breeding pairs were initially observed on a daily basis to monitor for signs of aggression, but once compatibility was determined, observations were more opportunistic and they were not disturbed for routine weighing. If tanks with young present were disturbed, adults occasionally killed their offspring, so in addition very little maintenance was done in these enclosures – minimal cleaning, no disruptive branch changing and generally reduced activity near the tank and in the room in general. On occasions, a female with a litter was not sighted for the duration of the period from birth to weaning. The males were generally more visible.

The best method of capture is to encourage the animal into a nest box, block off the entrance, slide one end of the box into a bag, and then remove the nest-box slide and drop the animal into the bag. Alternatively, a bag can be placed over the hand, the animal is then grabbed and the bag inverted over it. Hand-grabbing should only be attempted by experienced animal-handlers, as the tails of *P. fieldi* (as with most native Australian rodents) are very delicate, and any pressure or friction on them can result in a de-gloving injury to the tail (i.e. the skin slips off), leaving the vertebrae exposed. The exposed section of tail will usually dry up and fall off after some days.

## Aggression

In the first month of the programme (January 1997) there were two aggression-related deaths of paired animals (one male and one female in different pairs), one death in a group of five sub-adults, and the cannibalism of a litter by one of the parents. Two males were also attacked by the females they were paired with and were removed before serious damage was done. These incidents prompted the adoption of the husbandry procedures described in this paper, and a completely “hands-off” approach for enclosures containing young. Prior to this, larger groups of animals were held together, husbandry was more invasive and enclosures were less complex.

Watts and Aslin (1981) speculated that *P. fieldi* may be a loosely social species. In captivity this is certainly true to some extent for breeding pairs if sufficient space and cover are provided, but apart from a reproductively active pair, the only animals that could tolerate being housed together without becoming aggressive were litter-mates up to 60–70 days of age, and no more than four per tank. After this age, all the males must be removed and housed individually to avoid aggression, and the remaining females can generally remain together until approximately 90 days of age, when they too need to be separated and housed alone. Bite wounds from aggressive interactions have been found on the head, body and tail, and may lead to death if animals are not separated quickly.

Following these changes, the only other aggression-related deaths in the colony occurred when a male broke through overnight to an enclosure which held another male (he was euthanased after receiving critical injuries), and eight occasions where full or

**Table 1.** Typical birth intervals recorded for two *P. fieldi* females at Perth Zoo.

Female ID	Male ID	Date litter produced	Days between litters
199 (wild caught)	215	18 April 2000	
		15 May 2000	27
		12 June 2000	28
		14 July 2000	32
		16 August 2000	33
286 (captive born)	281	28 May 2001	
		30 June 2001	33
	342	15 November 2001	
		13 December 2001	28
		10 January 2002	28

partial litters of young were cannibalised, five of which occurred during an intensive “hands on” reproductive study (Butland 2001) towards the end of the captive programme.

### Reproductive parameters

Observations from the birth of 93 litters support the findings of Watts and Spencer (1978) that gestation in *P. fieldi* is approximately 28 days. Their data were from a single litter, produced 28 days after a copulatory plug was found, and the typical pattern of birth from this study indicates that *P. fieldi* are able to consistently produce new litters every 28 days or so. Table 1 shows the frequency of litter production for two female *P. fieldi*, which can be taken as representative of animals in the captive population.

Post-partum oestrus is common amongst the *Pseudomys* species (Happold 1976; Kemper 1976; Watts and Aslin 1981) and is also suggested in this study, with *P. fieldi* often giving birth every 28 days, the length of the gestation period established by Watts and Aslin (1981).

The youngest age for parents to produce a litter was 93 days, for both males and females. Taking into account a gestation period of 28 days, both sexes would have been sexually mature by at least 65 days of age.

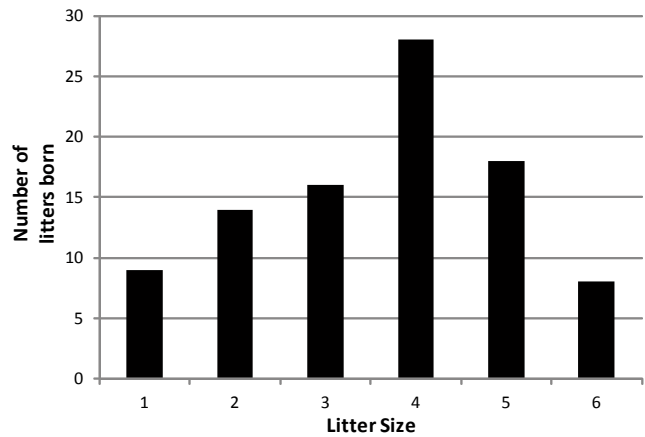
The oldest known age female to produce a litter (F286) was 625 days of age, and the oldest male (M307) was 531 days of age. The breeding programme was completed following these births and both animals were released, so it is possible that they may have continued to breed past this age. One male in this programme lived to 36 months of age. There are no data for life expectancy in the wild.

335 young were born between January 1997 and October 2002 (Figure 1), with an average litter size of  $3.6 \pm 1.43$  (mean  $\pm$  s.d.,  $n = 93$ , range 1–6).

In the wild, breeding has been observed to occur between May and November (Morris and Speldewinde 1992), but in captivity births were recorded all year round.

### Reproduction

When pairing animals for breeding it is important to follow the guidelines in the Housing and Aggression sections to ensure that enclosures are sufficiently complex and animals have the option

**Figure 1.** Litter size in *P. fieldi* at Perth Zoo.

to maintain separation from each other if desired. If a compatible pairing can be achieved, then reproduction can be maximised.

When pairs are introduced, aggression has been observed from either sex initially, but once a pregnancy has been achieved, it is generally the female that becomes the aggressor, particularly at parturition, when the male will be driven to attempt mating again in response to a post-partum oestrus. These behaviours have also been described for other *Pseudomys* species (Watts 1982). Signs of aggression may include constant chasing, an animal sitting out in the open, weight loss and bites on the tail and rump. If aggression becomes life threatening a pair may need to be separated but if introductions are carried out as described below this will not be common. The method was tried with good success following some early pairing deaths in the programme and so became the standard approach.

Each member of a breeding pair is provided with an aquarium of their own, and allowed at least a week to establish themselves before enclosure slides separating the tanks are removed to allow them to pass through at their own pace into each others' territory. If only one tank is available for a pair, then they should both be introduced at the same time to a clean enclosure, so neither has a territorial advantage. Each animal should be placed in the new enclosure in the nest-box from their previous enclosure (so that it retains their scent) so they have at least some small part of their own territory with them. Females were observed pulling leaves and sea-grass into nesting areas in the lead up to parturition, but if disturbed prior to giving birth would often vacate the nest. After birth they appeared more inclined to stay in the nest, but if they did flee, the pups were often taken along, as they attach to the teat and were commonly observed being trailed behind the female. This was regularly observed by Watts and Spencer (1978) until the pups were about 16 days of age. Newborn young could often be heard vocalising before they were sighted.

On one occasion, a male was observed moving his pups into a nest-box the day after the female had given birth. It was also a regular occurrence for males and newly weaned young to share a nest once the female had given birth to a new litter. The female would generally be in one tank with her new litter, while the male and the previous litter would occupy the second tank.

Young were weaned when the mother had given birth to a new litter i.e. at 28–30 days of age, at which point the pups generally weighed about 18–20 g. It was observed that young

naturally weaned themselves (moved away from the dam and into the territory of the sire) at about this age, so the 28–30 day weaning age was adopted as a routine husbandry protocol. It may be possible to remove pups at a younger age, but it was not considered important to determine this parameter, although on occasion, pups were successfully weaned at 16 g.

### Captive husbandry issues

In late November 1997 all breeding was suspended due to a temporary reduction in the requirements of animals for release, and when it became time to resume breeding in January 1999 there were difficulties in getting pairs to produce young. This is a reasonably common phenomenon amongst other native Australian rodent species such as *Zyomys pedunculatus* (Gaikhorst and Lambert 2009), and also *P. shortridgei*, *P. fumeus* and *P. australis* (A. Reiss, pers. comm.; W. Gleen, pers. comm.). As an arid-adapted species, it is likely that *P. fieldi* would respond to some kind of environmental cue, such as rainfall, to stimulate breeding, so when young are born there is food available for them to take advantage of. In captivity, those cues are very difficult to replicate, particularly when animals are housed indoors where living conditions and food quality are fairly consistent all year round.

Between 28 January and 6 February 1998, nine pairs were put together in an attempt to resume breeding, under the same husbandry and dietary regimes as when 19 litters were produced in the first five months of the previous year. In mid March sprouted seed was added to the daily diet, but by mid April, after no young had been produced, pairs were separated and most of the animals were released in May. In early June an attempt was made to simulate a seasonal change for the remaining three females and one male, whereby a lean dietary period was instigated (basic seeds and vegetables daily with rodent cubes added twice a week, and a starve day for animals held singly), followed by removal of the starve day towards the end of September and a significant increase in the quantity and variety of foods, including natural green leafy vegetation (salt-bush, pig-face, sea-spinach), sunflower seeds three times a week and the provision of seeding grasses on a daily basis. Green vegetation is a source of gibberellic acid, a plant hormone that may act to stimulate the onset of breeding (Towers and Halley 1996). The single male was paired on a rotational basis with each of the 3 females from early June until new stock arrived from the wild and five pairs were put together in late October.

By November, the male and one of the three females provided the lean diet had produced a litter, and then another in January. The other two females had litters very soon after, with one producing young in February and March, and the other in April and May. Unfortunately a control group was not used to test whether it was this strategy or some other cue that caused resumption in breeding, so should the opportunity arise in the future it would be useful to properly test this hypothesis.

### Releases

Three-hundred and forty-seven *P. fieldi*, of which 329 were captive bred animals, were supplied for release to the wild between 1997 and 2002. The Doole and North-West Island releases were managed by DPaW as highlighted in the *P. fieldi* Recovery Plan (Morris et al. 2000), and the zoo was engaged by the Australian Wildlife Conservancy (AWC) to provide animals for their Faure Island Sanctuary in Shark Bay (Morris et al. 2015; Seddon et al. 2015).

Prior to release all *P. fieldi* were familiarised with the kind of food items they would encounter at their release site. Most of those items (succulent vegetation and seeding plants) already formed

part of their regular diet, but if there was something that could only be sourced for a short period of time, they were provided with this for two weeks prior to release. Water was also restricted during this period (every second day) to further simulate conditions at release sites where there was no free water, and where vegetation would be an important moisture source. Animals were released once they were over six weeks of age and weighed at least 25 g.

### Discussion

In captivity, *P. fieldi* can breed all year round, and although breeding in the wild has only been recorded from May to November (Morris and Speldewinde 1992), it is possible that given the right conditions they may in fact be capable of breeding all year round in the wild as well. The ability to take advantage of good conditions and breed opportunistically is a typical pattern for arid-adapted species such as *P. albocinereus* (Happold 1976), which is also sympatric with *P. fieldi* on Bernier Island.

Jackson (2003) describes the behaviour of most *Pseudomys* species to be typically social, and Watts and Aslin (1981) suggest that the capture of a large number of male *P. fieldi* in a small area on Bernier Island (Robinson et al. 1976) may indicate that the trait applies to this species as well. Certainly in this and other studies (Watts and Spencer 1978; Butland 2001) some paternal care of young has been demonstrated; however, the housing of adult animals outside the bounds of a reproductive pair has met with too much aggression to be maintained, indicating that perhaps the social nature of the species may not be as developed as some other *Pseudomys* species such as *P. albocinereus*, *P. australis*, *P. apodemoides* and *P. chapmani* (Happold 1976; Cockburn 1981; Anstee et al. 1997), where the adults share communal burrows.

Husbandry procedures have been developed to enable the establishment of a productive captive colony of *P. fieldi*, provided that breeding is permitted to continue uninterrupted. The difficulties in bringing animals back into breeding condition following an enforced hiatus were significant, and although possible, this is not recommended if the goal of the programme is to maintain a high level of reproduction. We therefore suggest carefully planning the importation of founding stock from the wild during the peak of breeding and then permitting the animals to breed continuously. Opportunities to import new stock from the wild can often be limited (particularly when dealing with endangered species), so it is vital to manage these opportunities for the best possible outcome. As suggested by Gaikhorst and Lambert (2009) for a similar breeding programme for *Zyomys pedunculatus*, management euthanasia may need to be considered when there are resource considerations that may prevent uninterrupted breeding. At the very least, it is recommended that some pairs in a breeding room be permitted to continue breeding uninterrupted. It is postulated by the authors that the pheromones of reproduction may be sufficient to keep all animals in breeding condition, and further work to test this hypothesis would be useful.

Before the colony was disbanded and all animals released to the wild, it was made available for further study by Butland (2001), to determine whether the concerns regarding the impact of handling on reproductive success were valid, and to clarify some of the reproductive parameters. Results indicated that increased handling did not have an effect on any of the reproductive parameters, but did have a significant effect on the mortality of young through maternal aggression and cannibalism. This outcome was thought likely before the study began, and was the reason this research was not carried out concurrently during the early part of the breeding programme. However, these losses must be balanced against the valuable addition to our knowledge of reproduction and behaviour gained during the study.

There have been some positive results in the recovery of *P. fieldi*, with the species being downlisted from Endangered in 1991 to their current status today of Vulnerable (Department of the Environment 2014). The translocations to Faure Island and North-West Island have been very successful, with the North-West Island population even being used as a source population for further translocations to a mainland location in 2011 (Marmion 2011; Morris 2014; Australian Wildlife Conservancy 2013).

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